

Structure Function Studies of the SHV-1 Beta-lactamase

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DRUG RESISTANCE, MICROBIAL; BETA-LACTAMASES; BETA LACTAM RESISTANCE

SHV beta-lactamases are the second most prevalent group of beta lactamases found in Enterobacteriaceae. In collaboration with Dr. James R. Knox, we have crystallized SHV- I beta lactamase, SHV-1 beta-lactamase inactivated by the widely used inhibitor, tazobactam, obtained early crystals of SHV inactivated with clavulanate, and crystals of the extended spectrum beta-lactamase mutant, Gly238Ser. It was not until the crystal structures of the SHV-1 beta-lactamase were solved that a firm rationale for design and interpretation of structure-function studies became possible. Our goals are to understand what are the sequence requirements that confer resistance to oxyimino-cephalosporins and beta-lactam/ beta-lactamase inhibitor combinations in the SHV beta-lactamase and how these amino acid changes effect levels of expression. In this past two years, we constructed a total of 123 mutants. These include 18 mutations at 10 structurally conserved positions, all 19 possible mutations at Ambler positions Gly238, Asp104 (amino acids important in the expression of the ceftazidime resistant phenotype), Asp104Xaa-Gly238Ser, Ser130 (amino acid important in inhibitor resistance), mutations in the promoter region and Met69. We also are refining methods to precisely quantify beta-lactamase expression and are attempting to clarify the pathways of catalysis and inhibition in SHV by employing mass spectroscopy. The opportunity to combine molecular enzymology and microbiology permits us to ask probing questions regarding phenotype, beta-lactamase production and substrate and inhibitor specificity.

In this current proposal, we will extend our studies of the SHV beta lactamase and test the following hypotheses:

1. Asn170 is a critical residue in SHV: located in the omega loop, it plays an important role in the overall structure and function of the enzyme and plays a key role in hydrolysis of ceftazidime, a third generation cephalosporin.
2. The nature of the core amino acid residue at Ambler position 69 determines resistance to beta-lactamase inhibitors or to oxyimino-cephalosporins: the displacement of residues Ala237-Gly238-Glu240 on the b3 beta strand establishes the ceftazidime resistant phenotype. It is hypothesized that the Gly238Ser substitution results in resistance to third generation cephalosporins by displacement of the b3 beta strand. Since our last submission (Fall 2000), we determined that four amino acid substitutions at position Met69 confer resistance to ampicillin/clavulanic acid (Met69Ile, -Gly, -Leu, and -Val). The Met69Phe, -His, and, -Lys substitutions confer resistance to ceftazidime. This latter finding suggests a novel catalytic consequence to increased size and hydrophobicity of residues in this position.
3. Inhibitor resistant mutants of SHV are inactivated by an alternative mechanism than the susceptible SHV-1 beta-lactamase.

Our specific aims are as follows.

1. Site saturation mutagenesis of the Asn170 residues in wild type and Gly238Ser backgrounds. Perform minimum inhibitory concentrations (MICs) of each mutant in the wild type and Gly238Ser background, and determine steady state expression levels of the β - lactamase. Kinetic characterization the mutants that demonstrates the greatest resistance to oxyimino-cephalosporins will be performed.
2. Kinetic characterization of the beta-lactamase mutants at the Met69 position demonstrating the entire spectrum of beta-lactam resistance. We will purify the inhibitor resistant variants (M69Ile, -Gly, -Leu, and -Val) and the beta-lactamase mutant that confers the highest level of resistance to oxyimino cephalosporins (Met0fte). These studies will serve as a basis for explaining novel catalytic properties that will guide us to crystallization studies.
3. Determine the intermediates of inactivation of SHV-I and Ser130Gly beta-lactamase by inhibitors using mass spectroscopy.
4. Kinetic characterization and crystallization of the Ser130Gly mutant of SHV before and after tazobactam inactivation (Dr. James R. Knox).

It is clear that understanding the sequence determinants of resistance in SHV and the pathway to inactivation are important steps in the rational design and testing of future generations of beta lactam antibiotics and beta-lactamase inhibitors.